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Forum Review

Mitochondrial Uncoupling Proteins in the Central Nervous System

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ABSTRACT

Mitochondrial uncoupling proteins (UCPs), a subfamily of the mitochondrial transporter family, are related by sequence homology to UCP1. This protein, which is located in the inner mitochondrial membrane, dissipates the proton gradient between the intermembrane space and the mitochondrial matrix to uncouple electron transport from ATP synthesis. UCP1 (thermogenin) was first discovered in brown adipose tissue and is responsible for non-shivering thermogenesis. Expression of mRNA for three other UCP isoforms, UCP2, UCP4, and BMCP1/UCP5, has been found at high levels in brain. However, the physiological function(s) of UCPs in the brain have not been determined, although it has recently been postulated that UCPs regulate free radical flux from mitochondria by physiologically modulating mitochondrial membrane potential. In the CNS, this hypothesis has been studied primarily for UCP2. UCP2 message has been shown to be up-regulated in the CNS by stress signals such as kainate administration or ischemia, and overexpression of UCP2 has been reported to be neuroprotective against oxidative stress *in vivo* and *in vitro*, although the exact mechanism has not been fully established. In this review, studies on UCPs in the nervous system will be reviewed, and the potential roles of these intriguing proteins in acute and chronic diseases of the nervous system will be discussed.
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GENERAL INTRODUCTION TO UNCOUPLING PROTEINS

The electrochemical proton gradient (the proton motive force, $\Delta p = \Delta\psi + \Delta pH$) between the intermembrane space and the matrix of mitochondria is produced by the electron transport chain residing in the mitochondrial inner membrane (Fig. 1). As first proposed by Mitchell (61), this gradient is now known to be the driving force for ATP synthesis, called "coupling." Many proteins, including members of the mitochondrial carrier family, harness Δp for their function, some using its electrical component (ADP/ATP carrier and glutamate/aspartate carrier) and others using the ΔpH gradient (phosphate carrier and other carriers using substrate-H^+ symport). Any process using Δp *without* ATP synthesis has been termed "uncoupling." Uncoupling protein 1 (UCP1; thermogenin) was first recognized as the protein-mediated uncoupling activity in brown adipose tissues (BAT), in which it acts as the non-shivering heat generator to maintain the body temperature in newborns and hibernating mammals (9, 47, 64).

A number of other protein members of the SLC25 subfamily of the mitochondrial transporter superfamily (for review, see 67) have been designated as "uncoupling proteins" (UCPs) based initially on their sequence homology with UCP1 and evidence that they had many of the same functional motifs. Of those studied to date, all appear to be located in the inner mitochondrial membrane and to uncouple the proton gradient. More than 45 genes encoding UCP isoforms have been described in single-cell organisms, plants, and animals, including humans (54). In addition to UCP1, many of these additional UCP isoforms have been cloned from plants and mammals, and their physiological roles have begun to be investigated (8). UCPs are expressed in a tissue-specific manner, suggesting distinct roles and regulation of their function in different locations. UCP2 has broad tissue

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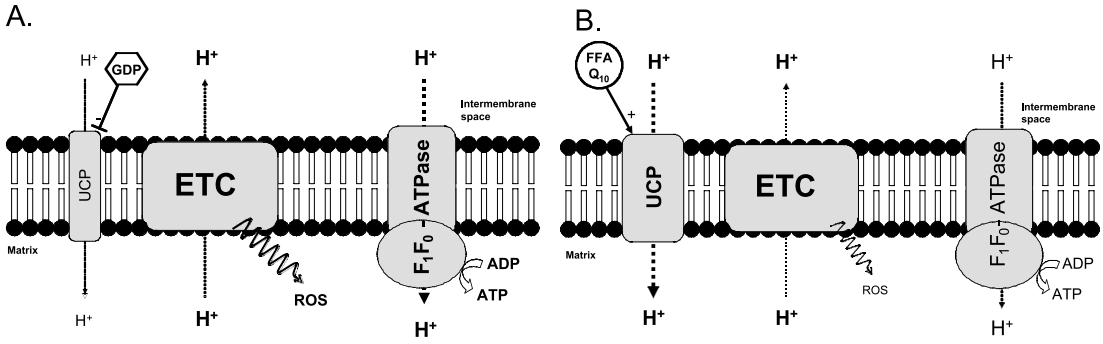

FIG. 1. Effect of UCP on the production of ROS. (**A**) Under normal conditions, the proton gradient established by the electron transport chain (ETC) is coupled to ATP synthesis through F_1F_0-ATPase. When the membrane is highly polarized, electron transfer from the ETC to oxygen is enhanced, increasing levels of ROS (superoxide, H_2O_2). In the presence of purine nucleotides (GDP), UCP activity is inhibited and elevated membrane potential and ROS production are maintained. (**B**) When overexpression of UCP or activation of the protein occurs, *e.g.,* by binding of free fatty acids (FFA) or coenzyme Q10, the proton gradient is brought below the critical point for ROS production, and mitochondrial ROS formation decreases.

expression, whereas UCP1 is almost exclusively expressed in BAT, and UCP3 is found mainly in BAT and skeletal muscle. UCP2 and UCP3 have the highest degree of homology to UCP1. Although they have significantly lower degrees of homology to UCP1, UCP4 and BMCP1/UCP5 have been added as putative members of the UCP family because they show most of the functional motifs of UCP1. Another isoform, SLC25A30, was recently reported by the RIKEN cDNA library to have a high degree of sequence homology to BMCP1/UCP5, but has not yet been studied. In BAT, UCP1 is highly expressed as one of the major mitochondrial proteins and, when activated, results in sufficient heat generation to maintain body temperature in newborns. Evidence suggests that UCP2 is *not* important for thermogenesis, however (2, 87). All UCPs are expressed at significantly lower levels in their respective tissues compared with UCP1 in BAT, suggesting that the function of these UCP isoforms may not be thermogenesis. The observation that the newer UCPs are expressed at such low levels prompted introduction of the concept of "mild uncoupling" (76)—in which functional regulation of mitochondrial reactive oxygen species (ROS) production occurs through modest decreases in Ψ_m—for the non-UCP1 family members (Fig. 1). Mitochondria are considered to be one of the main sources for intracellular ROS, and the mitochondrial membrane potential is the driving force for ROS generation as well as ATP production. A small decrease in Ψ_m can dramatically reduce production of ROS, specifically hydrogen peroxide (H_2O_2) (49). In addition, this mild uncoupling activity has been proposed to be one of the key modulators of cellular metabolism and signal transduction through regulation of mitochondrial ROS production (11, 32), because many critical signaling pathways have redox-sensitive components (30, 31). As the involvement of mitochondrial ROS dysregulation in neurodegenerative diseases has been a focus of research for many decades, and altered production or decomposition of ROS has been observed in many experimental models of CNS injury and neurodegenerative diseases, the possibility that UCPs, by regulating mitochondrial ROS levels, could be important modifiers of CNS damage has recently emerged. In this review, the involvement of UCPs in regulation of ROS will be described, and the physiological and pathological roles of UCPs in the nervous system will be discussed.

UCP EXPRESSION: TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL REGULATION

Although regulation of UCP gene expression at the mRNA level has been demonstrated in response to a diverse range of stimuli, including exposure to cold, high fat and ketogenic diets, starvation, leptin, insulin, inflammatory cytokines, and endotoxin (for review, see 14), regulation of protein is rarely reported. In part, this reflects problems with currently available UCP antibodies, including both custom-prepared and commercially available antibodies. Most are not exclusively specific to each isoform (69), and we have found that several antibodies detect 32–36-kDa bands in various knockout mice (Dugan and Kim-Han, unpublished observations), suggesting that specific, well characterized antibodies would be extremely useful reagents for future studies on the UCPs.

Regulation of UCP mRNA has not, in many cases, been followed by changes in UCP protein expression (43, 75), suggesting posttranscriptional regulation of UCP expression (23, 69). Recently, message for UCP2 has been found without concomitant protein expression (84). This disconnect between mRNA and protein expression results from an upstream AUG stop codon and alternative open reading frames (ORFs) in the 5′-untranslated region as a key determinant of posttranscriptional regulation (69). One or three upstream ORFs have been identified in UCP2/3 and BMCP1/UCP5, respectively, and these result in dramatically reduced efficiency of translation (Fig. 2). In addition, more than six mRNA splicing variants have been detected for BMCP1/UCP5 mRNA (Dugan and Kim-Han, unpublished observations). We have also experienced inhibition of UCP expression after *in vitro* transfection in mammalian cells, although mRNA was clearly present (Dugan and Kim-Han, unpublished observations). Other posttranscriptional mechanisms, including changes in mRNA stability, need to be studied for these pro-

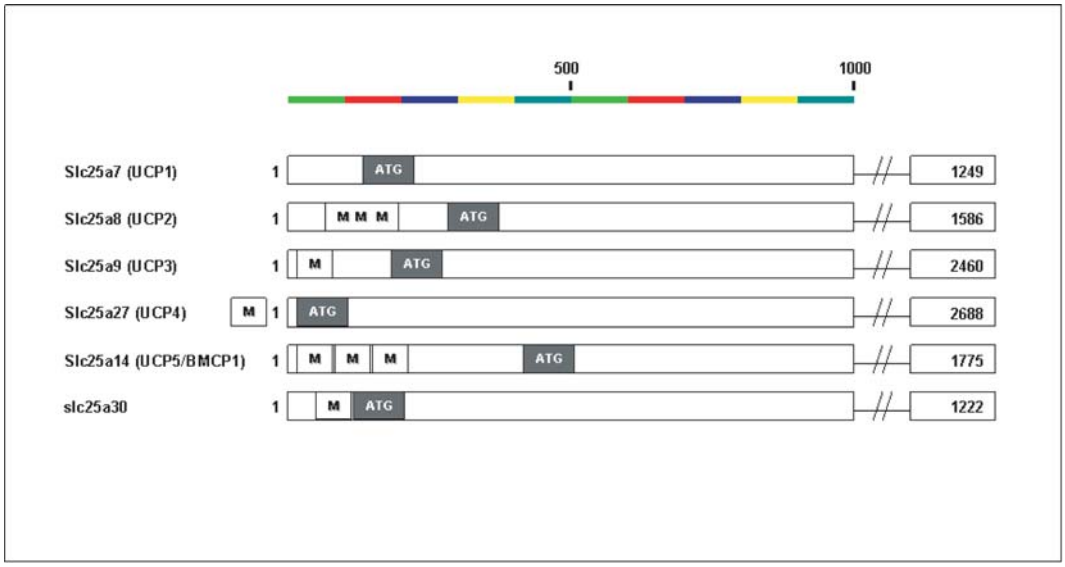

FIG. 2. The putative ORFs at the upstream of the starting codon in mouse UCP mRNAs. Among UCPs, UCP1 does not have any putative ORF. UCP2 and UCP5/BMCP1 have three ATG codons at the upstream of the starting codon with one and three ORFs, respectively. Rat UCP4 mRNA has an ORF found in the 5′-untranslated region. Slc25a30 is highly homologous to UCP5/BMCP1. (M, ATG codon. The size of mRNA is shown in the box at the right side.)

teins. In summary, it is important to bear in mind that the regulation of UCP mRNA expression may not correspond to that of protein expression, and that mRNA data as predictive of UCP protein or activity should be interpreted with some degree of caution.

REGULATION OF UCP ACTIVITY

Well known regulators of UCP activity are free fatty acids and purine nucleotides. Polyunsaturated fatty acids activate UCP, whereas bovine serum albumin, a fatty acid binding protein, inhibits UCP activity (25, 47). The purine nucleotides, ADP and GDP, also inhibit the proton conductance induced by various stimuli *in vitro* by binding to a specific allosteric site. The specificity and preference of a given UCP isoform for specific free fatty acid species has not been fully defined, but would be of interest because different fatty acid pools are released by varying stimuli, including ischemia and traumatic injury (1, 4, 36, 52, 53, 70, 79). In addition, there remains a controversy about whether free fatty acids are not only "activators" of UCP activity through a specific, high-affinity binding site, but "transported substrates" of UCPs, being cotransported with protons under selected circumstances (34). This alternative fatty acid transport activity has been most convincingly shown to date for UCP1 and UCP3. Regardless of their mechanisms of action, the requirement for nanomolar to micromolar concentrations of free fatty acids for full UCP activation has been well established (25, 35).

It was recently demonstrated that specific free radicals and oxidized macromolecules can modify the activity of UCPs. Echtay *et al.* reported that superoxide increases mitochondrial proton conductance through UCPs (26). This superoxide-mediated increase in conductance was sensitive to GDP and required fatty acids. Superoxide-stimulated proton conductance through UCPs was reversible, pH-dependent, and sensitive to superoxide dismutase (SOD), but not to catalase (26). Breakdown products of lipid peroxidation, such as 4-hydroxynonenal and its related compounds, specifically induce proton conductance of mitochondria through the uncoupling proteins (UCP1, UCP2, and UCP3) and the adenine nucleotide translocase in animals (27) and through StUCP (UCP homologue in *Solanum tuberosum*) in plants (77). Moreover, products of lipid peroxidation, such as cleaved hydroperoxy-fatty acids and hydroxy-fatty acids, have been reported to activate UCP2 and promote feedback down-regulation of mitochondrial ROS production (10).

Endogenously produced superoxide also increases proton conductance through activation of UCP3 in skeletal muscle, subsequently limiting mitochondrial production of superoxide (82). Recently, Krauss and colleagues reported UCP2 activation by superoxide introduced exogenously using mitochondria from kidney and spleen (50). Exogenously generated superoxide also activated UCP2 in thymocytes and B cells in mice, and activation was inhibited by MnTBAP, a SOD mimetic, or manganese superoxide dismutase (MnSOD), but not by glutathione peroxidase (GPX1). Although UCP activation by superoxide was absent in UCP2 knockout (KO) mice, implying that UCP2 may be activated by superoxide, in another study (18), UCP2 was clearly not activated by superoxide. As other UCP isoforms, specifically UCP1 (26) and UCP3 (26, 82), can be activated by superoxide, the expression of other UCPs in UCP2 KO mice should be determined to verify that other UCPs were not induced as has been shown previously (19). Specific activation of UCPs by superoxide also needs to be further confirmed by comparison with other inner mitochondrial membrane proteins. In addition, Li and colleagues pointed out that the superoxide-generating system, xanthine oxidase metabolism of xanthine, used for some of

these studies, consumes oxygen to produce superoxide, thus reducing oxygen content in the reaction mixture and modifying mitochondrial respiratory studies (56). Experiments using an *in vitro* reconstituted system might give more direct answers to this controversial question.

Ubiquinone has also been reported to be an activator of UCP1 (24). Short-term oral administration of ubiquinone induced nigral mitochondrial uncoupling and prevented dopamine cell loss after 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine administration in monkeys (41), although whether the effects of ubiquinone were due to activation of a UCP isoform, or to the multiple other actions (*e.g.* antioxidant) of this electron transport chain component, are not yet established. The requirement of ubiquinone for UCP activation has not been clearly demonstrated in other model systems, such as yeast and reconstituted liposomes (29, 42), so this remains an important area of UCP research.

Information regarding the regulation of UCP activity is likely to be more relevant than that of expression of UCP mRNA or protein *per se*. Why? With the exception of UCP1, expression of other UCP isoforms appears to be quite low, at least two orders of magnitude lower than that of UCP1, measured by [^{3}H]GDP binding (86). Furthermore, even in BAT, with its high content of UCP1, it is only after activation of UCP1 by fatty acids that uncoupling and thermogenesis can be detected, indicating that the presence of protein will not necessarily predict uncoupling activity. Experiments performed to determine UCP activity have generally been performed with isolated mitochondria from tissues that express mixed pools of UCPs, as well as other mitochondrial carrier proteins which may contribute to a small extent to "uncoupling" activity. Many studies using tissues and transfected cells have assigned uncoupling activity to a specific UCP without knowing the isoforms present in their systems. The contribution of one individual UCP in such preparations and the degree to which mitochondria "compensate" for the absence or overexpression of a single UCP remain an open question. Finally, the UCP expression profile in a given experimental model may differ from that reported in the literature. It is clear that there are strain differences in UCP expression even at baseline, and there are differing responses reported for the same treatment in different strains (37, 81).

MODULATION OF MITOCHONDRIAL ROS PRODUCTION BY UCPs

One of the main sites for intracellular ROS formation is the mitochondrial electron transport chain, in which superoxide is generated as a by-product of electron transport. In isolated mitochondria, inhibitors of complex I and III significantly increase the production of ROS (10, 13, 15). Production is closely dependent on mitochondrial membrane potential, *i.e.,* a small increase in Ψ_m can cause a robust production of superoxide and H_2O_2. A 10% decrease in Ψ_m resulted in a 55% decrease in H_2O_2 production (49). A 10-mV decrease in Ψ_m reduced superoxide production up to 70% by complex I following reverse electron flow from glycerol 3-phosphate (62). Physiological activation of UCPs has therefore been proposed to decrease ROS production. Although UCPs act as uncouplers, because of their low level of expression and the requirement for small-molecule activators for UCPs to be fully activated, the contribution of UCPs to regulation of Ψ_m is likely to be quite small, leading to the previously-mentioned concept of "mild uncoupling" (62, 76). This concept explains why there are no significant changes in basal levels of proton conductance in either UCP-overexpressing or KO animals until nonphysiological conditions are present. The role of UCPs in regulating ROS was first reported in 1997. In this study, GDP, an inhibitor of UCPs, increased both Ψ_m and production of H_2O_2 in mitochondria from BAT and liver (63). Macrophages from UCP2 KO mice produce more ROS, increasing their resistance to toxoplasmosis infection (2). Antisense treatment to decrease UCP2 expression increased ROS production and lipid peroxidation in murine endothelial cells (22). Mitochondrial H_2O_2 production is lowered by calorie restriction, and this appears to involve up-regulation of UCP3 in rat muscle mitochondria (7). Glucose-induced ROS production through early mitochondrial hyperpolarization was inhibited by UCP3 and UCP1 overexpression in dorsal root ganglia (DRG) neurons (83). In brain mitochondria, UCP2 overexpression also reduced mitochondrial ROS content, determined by flow cytometry using dichlorofluorescein fluorescence (59, 63, 66). Taken together, UCPs modulate ROS production possibly through decreasing mitochondrial membrane potential.

EXPRESSION AND FUNCTION OF UCPs IN BRAIN

Message for three UCP isoforms, UCP2, UCP4, and BMCP1/UCP5, has been found in brain. UCP2 mRNA is ubiquitously expressed in various tissues, including brain in rodents (38, 72), and is mainly neuronal, although there is some debate about whether UCP2 protein is expressed predominantly in macrophages under basal conditions (73). UCP4 message is exclusively in brain (58), whereas BMCP1/UCP5 is expressed in brain and several other tissues, including heart and kidney (45, 48, 74). Although no expression of UCP1 or UCP3 mRNA has been reported for brain, recently, UCP3 protein was found in rat DRG neurons immediately after dissection, but not in cultured DRG neurons (83). The authors suggested that UCP3 was protective against glucose-induced neuronal death in DRG neurons. Carefully performed quantitative RT-PCR analysis to determine the expression of UCPs in brain cortex indicated the following order: UCP5 > UCP4 > UCP2 >>> UCP3, UCP1 (55). Recently, expression of an isoform of BMCP1/UCP5 in fruit flies was also found predominantly in the head of adult flies (33), supporting an important role for this putative UCP in the CNS. The amount of overall UCP protein, shown by [^{3}H]GTP binding in brain, has been reported to be substantially lower than in BAT or spleen, but higher than in kidney, skeletal muscle, or liver (44). Despite the presence of at least three isoforms of UCP in brain, UCP2 has been the focus of the majority of studies on brain injury and neurodegenerative diseases. In normal brain, UCP2 mRNA expression is localized to specific brain regions, whereas in pathological conditions, UCP2 mRNA is

up-regulated in the regions affected by the injury. UCP2 mRNA is induced by transient ischemia in lesioned entorhinal cortex (5, 57) and in the periinfarct area in the neocortex and caudate putamen after 3–4 days (19). Both sublethal ischemia (59) and chemically induced seizures (21) increase mRNA expression in mouse hippocampus. Kainic acid (KA), given by intraperitoneal injection to mice, also induced UCP2 mRNA in the CA1 subfield of the hippocampus, the dorsal endopiriform nucleus, and the piriform cortex, areas sensitive to KA excitotoxity (6, 51). Induction was primarily neuronal and reached a maximum at 24 h, returning to basal levels within 72 h post injection. This neuronal induction of UCP2 mRNA by KA was more extensive in 129T2SvEmsJ, a KA-sensitive mouse strain, than in C57BL/6J, a KA-insensitive one, suggesting a specific link between UCP2 in KA excitotoxicity (17). Lipopolysaccharide, a commonly-used activator of oxidative stress, induced UCP2 mRNA, but not BMCP1/UCP5 mRNA in mice (12). Administration of a ketogenic diet increased mitochondrial respiration by fatty acids, possibly through induction of one or more uncoupling proteins (80). It has been postulated that ROS generation is one of the main factors for both neurodegeneration and aging, so UCP-dependent reduction of ROS in the nervous system has the potential to be neuroprotective in age-related neurodegenerative diseases, as well (28, 46).

Although UCP4 and BMCP1/UCP5 have sequence homology to UCP1, and UCP2–3 (29–34% and 34–39%, respectively) and show uncoupling activity in *in vitro* systems (58, 74), their function in brain has yet to be established. BMCP1/UCP5 mRNA was down-regulated by hypoxia and up-regulated by oxidative stressors, such as hyperoxia, 4-hydroxynonenal, or *tert*-butyl hydroperoxide in SH-SY5Y neuroblastoma cells (71). BMCP1/UCP5 message was decreased by chronic hypoxia and increased by transient global cerebral ischemia in rats (71), but was unaltered by cerebral ischemia in adult (19) and neonatal (Kim-Han, Reichert, and Dugan, unpublished observations) mice.

Among the three main isoforms of UCP expressed in brain, UCP2 is the most well characterized. UCP2 appears to reduce ROS production in brain under pathological, but not physiological, conditions. Focal cerebral ischemia produced by middle cerebral artery occlusion (MCAO) was used to test the role of UCP2 in CNS injury using both UCP2 transgenic and UCP2 KO mice. Both overexpressing and KO mice were resistant to ischemic injury (19, 59). Overexpression of UCP2/3 was neuroprotective against MCAO *in vivo* and oxygen-glucose deprivation *in vitro*. Levels of ROS in the mitochondrial matrix were lower in mitochondria from UCP2/3 transgenic mice than from nontransgenic controls, although there was no difference in total production of ROS (59, 68). In response to MCAO, UCP2 KO mice also showed a smaller infarct volume, less TUNEL-positive cells, no cytochrome *c* translocation, and less lipid peroxidation products compared with the control mice. The paradox that both overexpression and targeted deletion of UCP2 were neuroprotective has been explained by the observation that UCP2 KO mice show higher expression of MnSOD and had higher mitochondrial [GSH] than wild-type mice. Like the dicarboxylate and 2-oxoglutarate carriers (16), UCP2 may regulate mitochondrial GSH uptake. As UCP2 KO mice are presumed to produce more mitochondrial ROS, up-regulation of MnSOD and lowered consumption of GSH after ischemia have been proposed as compensatory mechanisms (19). These reports suggested that UCP2 can modify mitochondrial antioxidant defense systems. Overexpression of UCP2 also resulted in neuroprotection and lower caspase-3 immunoreactivity after entorhinal cortex lesioning (5) and was protective against oxidative stress-induced cell death in PC12 cells (21). Although UCP2 overexpression does not prevent seizures after scopolamine/pilocarpine treatment, it reduced cell death after the seizures (21). On the other hand, overexpression of UCP2 may cause cells to have difficulty providing enough ATP production for cellular metabolism, leading to mitochondrial biogenesis to support ATP production (40). In fact, UCP2 overexpression increased mitochondrial number, presumably to compensate for decreased ATP production by uncoupled mitochondria (21). In addition, UCP2 negatively regulates secretion of insulin (87) and dopamine (85) through an ATP-dependent process. How these effects factor into neuroprotection or neurodegeneration has not yet been determined, and may be complex.

Ethanol sensitivity was decreased in UCP2-overexpressing mice and increased in UCP2 KO mice. UCP2 also modified the impairment of pain and temperature sensation induced by ethanol, which is consistent with localization of UCP2 in primary sensory afferents of the spinal cord (38). Overexpression of UCP1 or UCP3 is also neuroprotective in glucose-induced neuronal cell death through glucose-induced superoxide production and glucose-induced mitochondrial hyperpolarization (83). Recently, the "uncoupling to survive" hypothesis was suggested by the positive correlation between metabolic rate and life span across many species (78). ROS production induced by oligomycin was significantly ($p < 0.05$) reduced in ketogenic diet-fed mice compared to *ad libitum* controls, suggesting that a ketogenic diet may have neuroprotective effects by diminishing ROS production through activation of mitochondrial UCPs (80), although the effects of ketogenesis on metabolism are quite complex, and include modulation of insulin signaling.

CONCLUSION AND PERSPECTIVES

UCPs are normally expressed in brain and regulated by CNS injury in affected brain regions, where they are postulated to cause mild uncoupling, reducing electron leakage from the electron transport chain, and thereby decrease superoxide generation. Mitochondrial ROS generation in pathological conditions is dependent on mitochondrial membrane potential, and despite a number of questions that remain to be answered, evidence suggests that overexpression of UCPs can be neuroprotective. However, in some situations, UCP activation may result in a balance between beneficial and detrimental effects. UCP expression can be proapoptotic, as well as antiapoptotic, depending on transcriptional and biochemical regulation (20, 60).

Mitochondrial membrane potential is the main regulator of mitochondria Ca^{2+} uptake, and many Ca^{2+}-dependent pathways are involved in neuronal cell death. Understanding the role of mild uncoupling in Ca^{2+} homeostasis and mitochondrial free radical generation in excitotoxicity may help our

understanding of a key mechanism of neurotoxicity (64). Finally, overexpression of UCP results in growth inhibition in yeast (3, 74), an effect that may be related not only to changes in energy metabolism such as reduced ATP production, but may also reflect dysregulation of redox-sensitive pathways, including activation of mitogen-activating protein kinase signaling.

The idea that this is due to modification of mitochondrial ROS production places these proteins in the intriguing position of integrating both metabolic function and oxidative stress. Although it is suggested that modification of UCPs can lead to many other cellular responses, including changes in antioxidant defense systems and mitochondrial biogenesis, careful investigation will be needed to identify the detailed mechanisms behind their neuroprotective effects. Elucidating the mechanism(s) of UCP action in the regulation of energy metabolism and ROS generation would advance our understanding of how cells regulate their "power plants" to respond to normal physiology and stress conditions.

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ABBREVIATIONS

BAT, brown adipose tissues; DRG, dorsal root ganglia; GSH, reduced glutathione; H_2O_2, hydrogen peroxide; KA, kainic acid; KO, knockout; MCAO, middle cerebral artery occlusion; MnSOD, manganese superoxide dismutase; ORF, open reading frame; ROS, reactive oxygen species; SOD, superoxide dismutase; UCP, mitochondrial uncoupling protein; UCP1, thermogenin, uncoupling protein 1.

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